Supporting Information

Degradable Optical Resonators as *In Situ* Microprobes for Microscopy-Based Observation of Enzymatic Hydrolysis

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1. General

Materials and Methods.

All reagents, solvents, and enzymes were purchased from Sigma–Aldrich, TCI, Acros Organics and Merck. Unless otherwise noted, all reagents and solvents were used as received. The aqueous dispersion of silk fibroin (6 wt%, pH = 5.0) was obtained from Nagasuna Mayu Inc. Optical microscopy (OM) and fluorescence microscopy (FM) observations were carried out using an Olympus model BX53 upright microscope. Scanning electron microscopy (SEM) was performed on a Hitachi Model S-3700N SEM operating at 20 kV. Ellipsometry measurements were carried out on a J. A. Woollam Japan Model M-2000 spectroscopic ellipsometer.

Microscopic PL (μ-PL) spectra were collected with a homemade microscope. equipped with a 405-nm cw laser and a spectrometer (Lambda Vision model LV-MC3/T, grating: 300 grooves mm^{-1} Fig. 1a). $\mathbf{MS_{silk}}$ was deposited onto a quartz substrate, and an individual $\mathbf{MS_{silk}}$ with an appropriate size and shape was selected based on the microscopic images, onto which the excitation laser was focused via an objective lens. The PL was collected via the same objective lens and transmitted to a spectrometer via an optical fibre.

Synthesis of microspheres from fibroin proteins

In a typical procedure, an aqueous dispersion of silk fibroin proteins was mixed with Span 80 as a nonionic surfactant and stirred vigorously with a homogenizer. Then, the dispersion was centrifuged and washed with EtOH to remove the excess Span 80. The resulting powder was dispersed in a DMF solution of Nile Red and stored at 85 °C for 6 h for doping. After cooling to 25 °C, the dispersion was centrifuged twice to remove the residual dye, yielding fluorescent $\mathbf{MS_{silk}}$.

Synthesis of microspheres from albumin

An albumin dispersion was prepared by adding 50 mg of crude albumin from dried egg white (TCI, Japan) into 1 mL of water. The mixture was then sonicated to form an albumin dispersion with a concentration of 50 mg mL^{-1}. Two millilitres of albumin solution (50 mg mL^{-1}) was fed into an airbrush with regulated pressurized gas (0.1 MPa). Into a PFA beaker filled with a 50 mL mixture of AcOH and BuOH (4.8/100 *v*/*v*), the albumin solution was directly sprayed. The resulting suspension was incubated at 25 °C for 6 h. The albumin microspheres were retrieved, centrifuged and washed three times with EtOH. The powder was then dried at 25 °C for 1 d.

Dyeing of microspheres from albumin, potato, and wheat starch

We conducted the dyeing process according to our previous report.[18] The powdery specimen of the microspheres (1 mg) was dispersed in a DMF solution of Nile Red (1 mL, 300 μg mL^{-1}), and the dispersion stood at 85 °C for 6 h. After cooling to 25 °C, the microspheres were collected and centrifuged twice to remove the excess dye.

Reaction kinetics analysis based on weight

A dispersion (1 mL) containing the microspheres (10 mg) and the catalyst was incubated at 25 °C on a shaker. After a certain interval, the dispersion was centrifuged in a microtube to remove the supernatant. The residuals were dried under vacuum. The weight of the solid was measured with a balance.

Equations for the TE and TM modes of the WGM resonance peaks

The WGM resonance contains two polarization-dependent modes, namely, transverse electric (TE) and magnetic (TM) modes. The TE and TM modes satisfy the equations below:

$$\lambda_n^H = 2\pi r(\varepsilon\mu)^{\frac{1}{2}}\left[(n+\frac{1}{2})+1.85576(n+\frac{1}{2})^{\frac{1}{3}}-\frac{1}{\mu}\left(\frac{\varepsilon\mu}{\varepsilon\mu-1}\right)^{\frac{1}{2}}\right]^{-1} \quad \text{(Eq. 3)}$$

$$\lambda_n^E = 2\pi r(\varepsilon\mu)^{\frac{1}{2}}\left[(n+\frac{1}{2})+1.85576(n+\frac{1}{2})^{\frac{1}{3}}-\frac{1}{\varepsilon}\left(\frac{\varepsilon\mu}{\varepsilon\mu-1}\right)^{\frac{1}{2}}\right]^{-1} \quad \text{(Eq. 4)}$$

where λ_n^E and λ_n^H are the wavelengths of the n-th TM and TE mode PL, respectively, ε (= η^2) is the dielectric permittivity, μ (= 1) is the magnetic permeability, and r is the sphere's radius. Here, the much higher order term was neglected.

Calculation of the relative change in volume from the relative change in wavelength

The relative change in volume was calculated based on the relative change in the wavelength of the resonance peaks. Given that the changes in volume, diameter and wavelength were small in comparison to their original values,

$$\Delta\lambda/\lambda = \Delta d/d \quad \text{(Eq. 5)}$$

according to a previous report.[24] In addition, the relative change in volume can be described as

$$\Delta V/V_0 = 3\Delta d/d \quad \text{(Eq. 6)}$$

which was obtained by the differential calculation of the equation of volume. By combining Eq. 5 and Eq. 6, we obtain

$$\Delta V/V_0 = 3\Delta\lambda/\lambda \qquad \text{(Eq. 7)}$$

Thus, the remaining volume V relative to the original value can be expressed as

$$V/V_0 = 1 - 3\Delta\lambda/\lambda \qquad \text{(Eq. 7)}$$

2. Supporting Figures

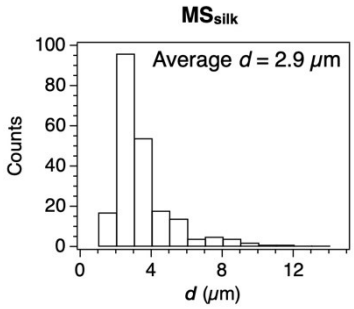

Fig. S1. Histograms of the size distribution of $\mathbf{MS_{slik}}$ calculated based on SEM images.

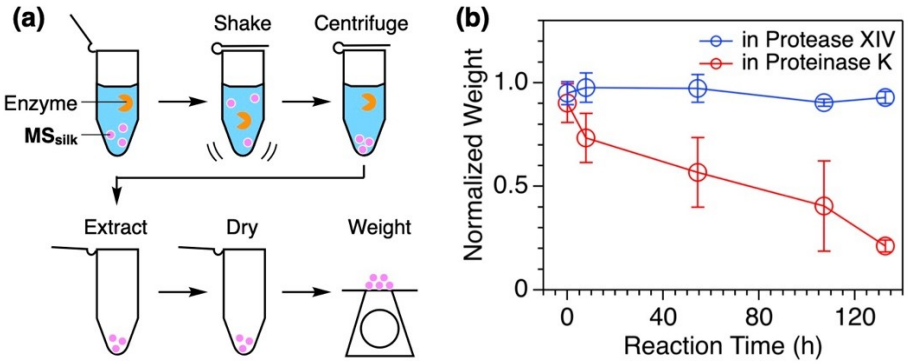

Fig. S2. (**a**) Schematic illustration of the weighting method for measuring the kinetics of the hydrolysis reaction $\mathbf{MS_{silk}}$. (**b**) A plot of the changes in normalized weight of the solid components in aqueous dispersions of $\mathbf{MS_{silk}}$ after the incubation with Proteinase K (red) and Protease XIV (blue).

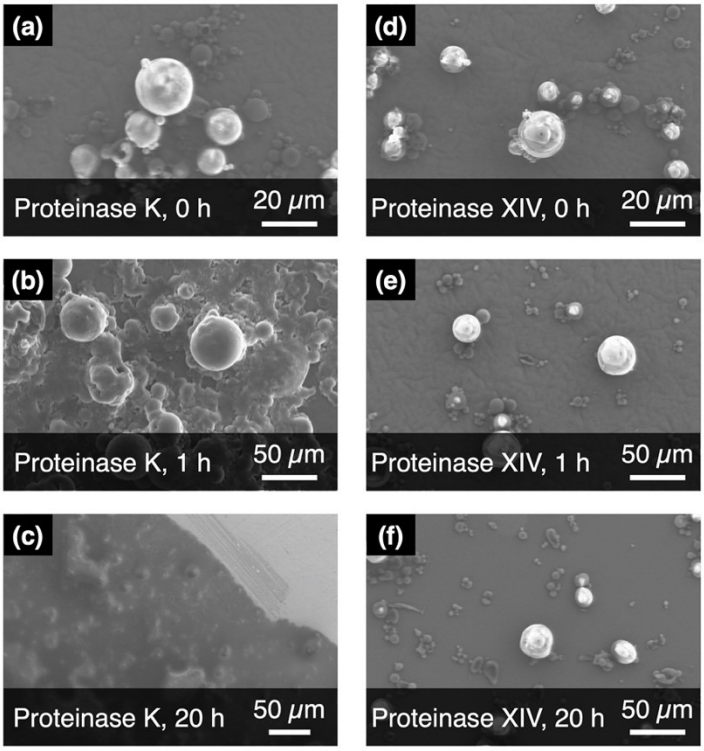

Fig. S3.SEM images of $\mathbf{MS_{silk}}$ after incubation in an aqueous solution containing an enzyme.

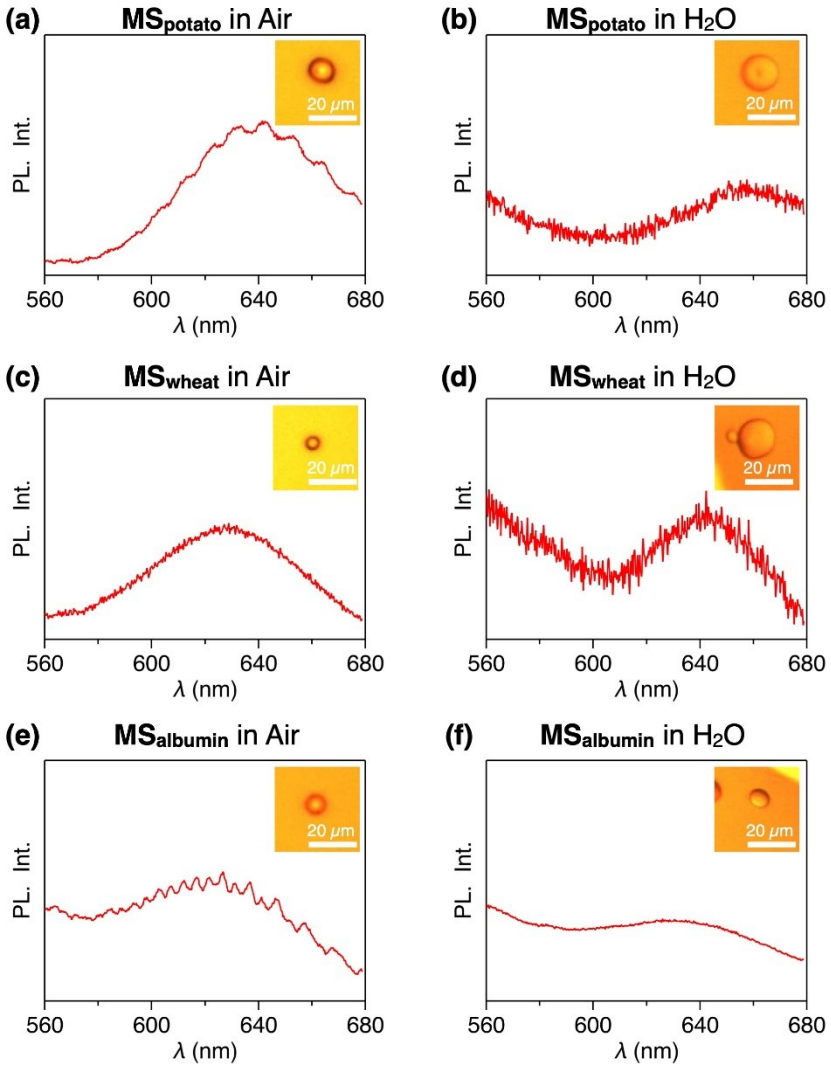

Fig. S4. μ-PL spectra of $\mathbf{MS_{potato}}$, $\mathbf{MS_{wheat}}$, and $\mathbf{MS_{albumin}}$ observed in air and water, respectively. The insets show the microscopic images of the particles.

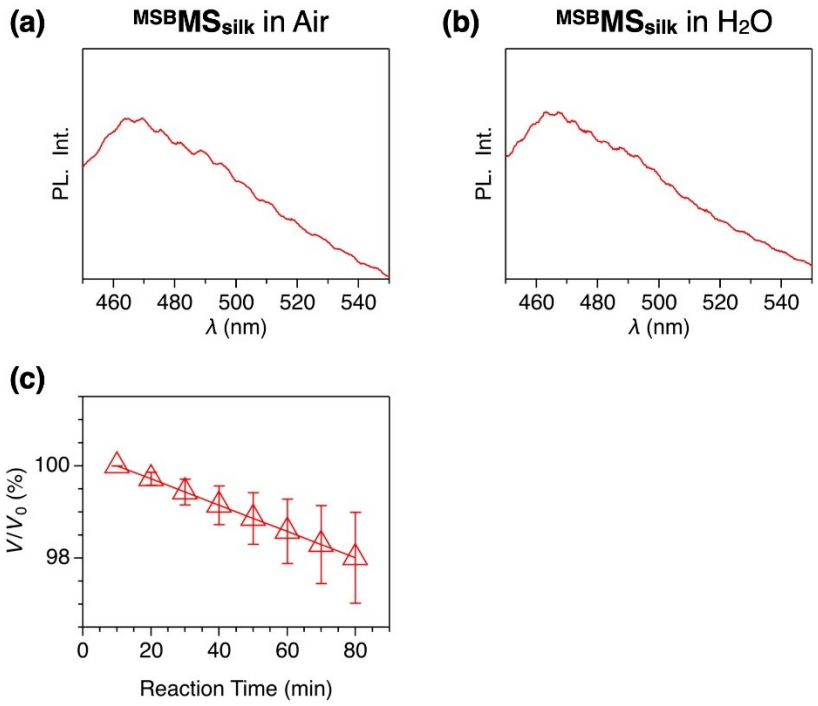

Fig. S5. (**a,b**) μ-PL spectra of $^{MSB}\mathbf{MS}_{silk}$ observed in air and water, respectively. (**c**) A plot of the relative change in volume with error bars (one sigma) calculated based on the spectral shift of $^{MSB}\mathbf{MS}_{silk}$.